The mariculture potential of two carrageenophyte species, *Iridaea capensis* J. Ag. and *Gigartina teedii* (Roth) Lamouroux, on the South African West Coast

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University of Cape Town
1995

3317BB & 3318AA SALDANHA

1:50 000 SUID-AFRIKA. SOUTH AFRICA

18°00'

Hoedjiesbaai
Pepper Bay
SALDANHA
Smitswinkelbaai
Noordbaai of Brandewynbaai
Hoedjiespunt
Bomgat
Ore Jetty
Ibaai
Skulpiesbaai
Entrance Channel
Marcus Island
Hospital Rock
Papheuwel
FARM 195
Spreeuwal
FARM 949
OS FONTEIN 194
OLIFANTS KOP
UYEKRAAL 189
Kleinberg
Tiekosklip
Elandspunt
Boat Rock
Salamanderpunt
Salamander
Salamanderbaai
Stormbaai
Camp Point
Donkergat
Juttenellland
Meeueiland
Juttenbaai
Rietbaai
Skaapeiland
Perlemoenpunt
Juttenpunt
Hugospos
Stormbaai
Luisterhoek
South Head
LYFSERSHOEK
NIEUWLAND
Drie Susters
Plankiesbaai
POSTBERG NATUURRESERVAAT
OUDE POST 367
Stony Head
KREEFTE BAAI 368
Kreefbaai
Kreelbaai
Klein Eiland
Vondelingeiland 369
LANGEBAAN
Leentjiesklip No. 3
Leentjiesklip No. 2
Leentjiesklip No. 1
Olifantskop
Bontheuwel
Groot Diamant
Tentklip
Septemberskip
Hartbeesrug
Evergreen Villa
Landingstrook
Meeuwklip
Meeuklip
Panorama
Stompneusrots
Japan se Klip
Klein-Oosterwal
Oosterwal
Sondagsheuwel
WASCHKUIL
STSKOP
Seeberg
Die Brak
Flemming's Point
Kliphoek
Salpetersvlei
DIE LAGUNE
Langebaan Lagoon
Kraalbaai
Preekstoel

ABSTRACT

In an attempt to propagate the carrageenophyte species *Gigartina teedii* and *Iridaea capensis* vegetatively on rafts in Saldanha Bay, only the *G. teedii* showed potential using this mariculture technique. The carrageenan contents of the gametophyte and tetrasporophyte generations of *G. teedii* were found to be 35.4% & 39.7% respectively. The resorcinol test showed that the Kraalbaai population of *G. teedii* was 23.7% tetrasporophyte. The optimal light intensity for *I. capensis* sporeling growth proved to be much higher (over 50μE.m^{-2}.sec$^{-1)}$ than found for most other species. Photosynthetic rates of *G. teedii* and *I. capensis* thalli were measured over a range of light and temperature regimes, in order to determine optimal conditions for these two species. The P-I curves were similar for the two species, with *I. capensis* and *G. teedii* having *Ik* values of 230.9 & 206.9 μE.m^{-2}.sec^{-1} respectively. The optimal temperature for photosynthesis of *G. teedii* was higher (20°C) than that of *I. capensis* (15°C). The results obtained in this study are used not only to assess the mariculture potential of the two species in question, but also to propose guidelines for the screening of other South African carrageenanophytes with respect to their mariculture potentials.

Introduction

This project had its beginnings in the 1994 International Seaweed Symposium held in Chile. Members of two of the largest carrageenan manufacturing companies, FMC and Copenhagen Pectin, expressed an interest to Dr John Bolton of UCT and Dr Rob Anderson of Sea Fisheries in the potential role of South Africa in the carrageenan industry.

The modern carrageenan industry dates from the 1940's, receiving its impetus from the dairy industry where carrageenan was found to be the ideal stabilizer for the suspension of cocoa in chocolate milk (Chapman 1980). Since then, dozens of new food and pharmaceutical applications have been discovered for carrageenan, such as its inclusion in toothpaste and tinned meats. As a result, the present annual worlwide production of carrageenan is 15,000 tonnes, worth a total of US$115,000 (Luning 1990, cited in Critchley 1993).

Carrageenan is a primary constituent of the cell walls of certain red algae. Like the other phycocolloids, carrageenan serves a structural function analagous to that of cellulose in land plants. Whereas land plants require a rigid structure capable of withstanding the constant pull of gravity, marine plants must have a more flexible structure to accommodate the varying stresses of currents and wave motion. Thus, they have developed hydrophilic, gelatinous structural materials with the necessary flexibility. For this reason, carrageenans, as well as alginates and agars, possess unique functional properties that enable them to thicken, gel, emulsify, and stabilize many food

and industrial products.

There are three main types of carrageenan, *kappa*, *lambda*, and *iota*, each having its own gel characteristics. Various red algal species may contain one or more of the different types of carrageenan, and the refined product is tailor-made for specific applications by blending certain species in precise quantities. Since the invention of the technique for the mariculture of *Eucheuma* spp. in the 1970's, the carrageenan industry has been dominated by *Eucheuma* cultivated in the Philippines, Indonesia and, more recently, Tanzania. *Eucheuma* spp. now account for 79.9% of the harvested tonnages of carrageenophytes on a global scale (McHugh 1991).

But although *E. cottonii* and *E. spinosum* (the most widely maricultured species) yield good *kappa*- and *iota*-carrageenan respectively, there are certain other types of carrageenan, such as *lambda*, which are absent in this tropical genus. Thus, the harvesting of certain cold-water carrageenophytes from natural stocks is still a necessity. For example, *Chondrus crispus* is harvested in Canada and France for its *lambda* carrageenan in the tetrasporophyte generation, while tetrasporophytic *Gigartina* and *Iridaea* from South America and southern Europe are excellent sources of *lambda* carrageenan (Critchley 1993).

In the 1970's, research was conducted on the mariculture potential of several cold-water carrageenophytes, particularly *Chondrus crispus*, *Gigartina exasperata* and *Iridaea cordata*, off the east and west coasts of the North American continent. Although initial growth rates looked promising, it was most likely the financial constraints imposed by high material and

labour costs that prevented the success of these enterprises. For example, a strain (*T4*) of *Chondrus crispus* was found that could propogate vegetatively in flowing seawater (Simpson *et al.* 1979). But since expensive tanks with continuously pumped seawater were required for this method of cultivation, the profit generated from sales could never cover the running costs.

The fact that no viable technique for the mariculture of cold water carrageenophytes has been developed, has led to a relative shortage of this resource in comparison to the successfully maricultured tropical *Eucheuma* spp. This fact is evident from the current FOB prices of carrageenophytes: US$ 1000 for a dry ton of *Gigartina/Iridaea*, in comparison to US$ 700 for *Eucheuma* spp. (C. Dawes, pers. com.). If the present demand for cold water carrageenophytes continues to rise, overharvesting of natural populations is likely to take place, in the same way that Asian *Eucheuma* stocks were depleted in the late 1960's (Doty & Alvarez 1975). It is for this reason that the leading carrageenan manufacturers are searching for new sources of cold water carrageenophytes. Moreover, the booming economy of Chile, the major exporter of *Gigartina/Iridaea*, has meant that this country is demanding higher prices for its carrageenophytes due to the rising standard of living of those involved in seaweed harvesting (R.J. Anderson, pers. com.).

But what role could South Africa play in the world carrageenan industry? A number of species containing carrageenan occur along the South African coastline, and many of these species belong to genera that are harvested elsewhere in the world. Although none of these carrageenophytes are currently

being harvested in South Africa, up to 54 tonnes per annum of *Gigartina polycarpa* (probably including *G. stiriata*) were harvested from 1956 to 1978 (Anderson *et al.* 1989). Yet this figure is hardly comparable to the 5-6,000 tonnes of *Iridaea* harvested annually in Chile (Santelices 1989). The reason for this is that South African carrageenophyte populations are either too sparse, too scattered, or inaccessible (Anderson *et al.* 1989).

Therefore, if South Africa wishes to enter the carrageenan market, natural stocks will not suffice, making mariculture a necessity. Although studies on tank cultivation of seaweeds have shown rapid growth rates, high construction and maintenance costs have prevented the commercial acceptance of this method. On the other hand, the use of ropes as artificial substrates for seaweed attachment is widespread in both the seaweed food and phycocolloid industries (Critchley 1993).

There are two major approaches to the mariculture of seaweed on ropes. The first involves seeding the ropes with spores from fertile adult plants, and is the dominant method used in the production of seaweed for food in the Far East. For example, species of the genera *Monostroma, Enteromorpha, Laminaria, Undaria, Cladosiphon* and *Porphyra* are all maricultured by seeding ropes in tanks, and the ropes are then transferred to rafts in the sea. The second approach to seaweed mariculture involves attaching vegetative fragments of adult thalli to ropes in the sea. This technique has proved to be extremely successful in the mariculture of *Eucheuma* and *Gracilaria*, since species of these genera are capable of vegetative reproduction.

In this project, the mariculture potential of two carrageenan-containing species has been investigated, namely *Iridaea capensis* J. Ag. and *Gigartina teedii* (Roth) Lamouroux. These two species are members of the Gigartinaceae, which have *lambda*-family carrageenan in the tetrasporophyte generation, and are therefore of potential economic value. Both species have a typical red algal, triphasic life history, with an alternation of isomorphic haploid and diploid phases. They begin growth following spore germination as a prostrate crust which then produces upright plants.

Iridaea capensis is a generally unbranched, strap-like alga that is common from Cape Agulhas westward into Namibia (Bolton & Joska 1993). Biomass surveys have revealed that populations of this species are too scattered to be economically useful, especially because its distribution is almost entirely confined to areas of rocky shore directly affected by sand action (Bolton & Levitt 1992). Population studies in the south-western Cape Province revealed that carrageenan levels were highest (42% of dry weight) in late winter-spring, and lowest (30-35%) in summer-august (Bolton & Joska 1993). Because of *I. capensis'* blade-like morphology, it is highly unlikely that it can be grown vegetatively. Therefore, the possibility of mariculturing this species using the sexual approach, i.e. seeding ropes with spores from fertile blades, was investigated.

Gigartina teedii is a relatively uncommon alga of the intertidal and shallow subtidal in the Mediterranean and Atlantic (Guiry *et al.* 1987). It occurs in sheltered areas on both the south and west coasts of Southern Africa, with collections having

been made at Rocky Point (Namibia), Saldanha Bay, the Kowie River, and Algoa Bay (Bolus Herbarium). Due to its multiaxial structure, it may be possible to multiply this species vegetatively, and for this reason *G. teedii* was selected as a suitable candidate for the asexual mariculture method.

Materials and Methods

MARICULTURE EXPERIMENTS

In February 1995 (late summer) an attempt was made to grow *G. teedii,* and *I. capensis* vegetatively on ropes at two different locations in Saldanha Bay. The ropes were attached to two rafts that have been been constructed in Saldanha Bay for the purpose of seaweed mariculture experiments on the agarophyte *Gracilaria verrucosa*. One of the rafts, situated centrally in the bay, belongs to Sea Fisheries, while the other raft was constructed on the western side of the bay by Sea Harvest. The *G. teedii* plants were collected at Kraalbaai (on the western side of Langebaan Lagoon), while the *Iridaea* plants came from St. Helena Bay. Six ropes (replicates) for each species were prepared by attaching 20 plants 15cm apart on 3m long ropes by threading the stipe of *I. capensis* and the main axis of *G. teedii* through the ropes. Three ropes of each species were then suspended at a depth of roughly 0.5m on both of the rafts, and left for 2 months. Repeat experiments were performed in July (winter) with *G. teedi* and in August with *I. capensis*.

G.TEEDII EXPERIMENTS

Ratio of Life History Phases

G. teedii was collected at Kraalbaai in February. The plants, which occurred from just above MLLW to a depth of approximately 1m at low tide, were picked randomly, and the total biomass was weighed. Since the female gametophyte plants were easily identified by the presence of cystocarps, they were separated and weighed. Of the remaining plants, 96 were randomly screened by the resorcinol test, a staining test which separates plants with *kappa* and *lambda* carrageenan (Craigie & Leigh 1978). In this way, it was possible to estimate what percentage of the remaining plants was male gametophyte and what percentage was tetrasporophyte, and these figures were used to determine the ratio of gametophytes to tetrasporophytes for the initial sample.

Because *G. teedii* material had to be collected at Kraalbaai a number of times over the course of the year, several observations were made concerning the population dynamics of this species at this site.

Carrageenan Content

Using the *G. teedii* plants collected at Kraalbaai in February, the carrageenan contents of 12 plants, 6 from each life history phase, was determined by the method of Santos & Doty (1975). This is a laboratory water extraction method, with precipitation using iso-propanol. The only modification was the use of centrifugation rather than pressure filtration for separation (Bolton & Joska 1993).

IRIDAEA EXPERIMENTS

Sporeling Growth Rates

Fertile *I. capensis* blades were collected from Wireless Road, Kommetjie, on the west coast of the Cape Peninsula. The plants were scrubbed, rinsed, wiped dry and placed in 10°C overnight. The following day, contaminating organisms were cleaned from the surface by a 20 sec. immersion in 0.5% bleach (Jik) in seawater. The plants were again rinsed, and then placed in seeding buckets containing filtered, autoclaved seawater at 15oC. Sixteen microscope slides had been arranged so as to cover the bottom of the buckets. After 4 hours the blades were removed from the buckets. After 24 hours each of the 16 seeded microscope slides was placed in a petri dish containing filtered, autoclaved seawater plus one third Provasoli's Enriched Seawater medium (PES; Stein 1973). The petri dishes were divided into four sets of 4, and each set was placed in one of four different light intensities in a room with a constant temperature of 15°C. The four light intensities (5, 20, 35 and 50 $\mu E.m^{-1}.sec^{-1}$) were obtained by placing the petri dishes at different distances from a light source (five parallel cool, white flourescent lights). The seawater and PES was changed once a week, and the growth rates were determined by measuring 100 spore diameters for each light intensity (25 for each of the 4 microscope slides) after 12, 25 and 35 days.

Seeding Ropes

The temperature-shock method of spore release was again used, but instead of microscope slides, a coiled 3m length of

polypropylene rope was placed at the bottom of each of four seeding buckets. The fertile blades were removed from the seeding buckets after 4 hours, and after 24 hours the seeded ropes were transferred to 20x20x15cm glass tanks, containing filtered seawater plus one third PES. The tanks were then kept at 15°C and at a light intensity of 35 $\mu E.m^{-2}.sec^{-1}$, with seawater being replaced on a weekly basis. After 3 months the *I. capensis* sporelings had produced blades up to 1.6mm in length, and the ropes were transferred to the Sea Harvest raft in Saldanha Bay.

LABORATORY CULTURE EXPERIMENT

An attempt was made to study the growth rates of *I. capensis* and *G. teedii* over a range of light intensities. Since winter is the recruitment period for *I. capensis* (Bolton & Joska 1993), it was possible to collect a large number of juveniles of this species at Wireless Road, Kommetjie, in July. Plants from 10-30mm in length were placed in petri dishes containing filtered, autoclaved seawater and placed in a range of light intensities at 15°C. After a few days the majority of the plants had begun to die, with the infection setting in in the region of the holdfast and stipe. For this reason the experiment had to be terminated.

A similar experiment was attempted with *G. teedii* using excised tips instead of juvenile plants. However, after 5 days the tips began to lose their colour and die, possibly because the plants were collected from Kraalbaai in July, a time of the year when the plants at this site had begun to die on a large scale. Since the *G. teedii* population only started to recover at the end

of August, time pressure necessitated that the growth experiments be replaced by photosynthesis experiments over a range of temperature and light conditions.

PHOTOSYNTHETIC RATES

Pieces of *G. teedii* thalli weighing approximately 2 grams were placed in incubation bottles containing deoxygenated filtered autoclaved seawater. In the case of *I. capensis*, juvenile plants weighing as close to 2 grams as possible were used. The oxygen concentration of the seawater was reduced by bubbling N_2 (gas) through it for 10 mins to prevent inhibition of photosynthesis due to oxygen supersaturation. Three incubation bottles (250ml) for both species were kept for 90 minutes in a 15°C room at each of five different light intensities (40, 100, 150, 225, & 300 $\mu E.m^{-2}.sec^{-1}$). Preliminary calculations revealed that the optimal light intensities for *G. teedii* and *I. capensis* were 150 and 225 $\mu E.m^{-2}.sec^{-1}$ respectively. Therefore, three incubation bottles for each species were kept for 90 minutes in each of the five temperature regimes (10, 15, 20, 25 & 30°C) in 190 $\mu E.m^{-2}.sec^{-1}$ (i.e. the approximate average of the optimal light intensities of the two species). Two dark incubation bottles were also kept at each of the five temperatures for 90 minutes. A YSI Oxygen Analyser was used to measure the initial and final oxygen contents of the light and dark incubation bottles.

The intersection of the light-limited regression lines of the 2 P-I curves with a line drawn parallel to the abscissa and through the point of maximum net photosynthesis was employed to statistically estimate light saturation (*Ik*). Net photosynthetic

rates are given in terms of mg O_2 fixed.dry $wt^{-1}.h^{-1}$. When it was necessary to compare a reading to another publication that expressed its data in mg C. g dry wt^{-1}. h^{-1}, the conversion from mg O_2 to mg C was performed by assuming a photosynthetic quotient of 1.2, and a respiratory quotient of 1.0.

STATISTICAL ANALYSES

All data analyses were executed on a statistical software package, Statgraphics v5. The Mann Whitney-U Test was used to test for significant differences between pairs of data sets with small sample sizes. When multiple sets of data were being compared, the Kruskall-Wallis one way ANOVA was performed. Where this test showed significant differences, these were revealed by multiple range testing using LSD in a multifactor ANOVA.

Results

MARICULTURE EXPERIMENT

The *G. teedii* placed on the rafts in February not only survived but also increased significantly ($p<0.05$) in biomass over the 59-day experiment, as can be seen in Tables 1 & 2. The difference between the increase in biomass at the two sites proved not to be significant ($p>0.05$). However, the increases in weight cannot be attributed purely to growth of *G. teedii*, since the exposed areas of rope between plants became overgrown with epiphytic species of *Enteromorpha* and *Ceramium*. Despite the fact that the *G. teedii* plants appeared larger and reasonably healthy at the end of the experiment, they were also covered by

TABLE 1.

GROWTH OF *G.TEEDII* AT SEA FISHERIES RAFT			
	SEAWEED WET WEIGHT (9 FEBRUARY)	SEAWEED WET WEIGHT (9 APRIL)	% CHANGE IN BIOMASS
ROPE 1	125g	305g	248%
ROPE 2	195g	200g	211%
2ROPE 3	110g	260g	236%

TABLE 2.

GROWTH OF *G.TEEDII* AT SEA HARVEST RAFT			
	SEAWEED WET WEIGHT (9 FEBRUARY)	SEAWEED WET WEIGHT (9 APRIL)	% CHANGE IN BIOMASS
ROPE 1	100g	220g	220%
ROPE 2	90g	250g	278%
ROPE 3	80g	245g	306%

TABLES 1 & 2. The change in wet weights of 20 *G.teedii* plants attached to 3 ropes on each of the Saldanha Bay rafts over 2 months in late summer.

a film of diatoms. It is therefore plausible that these plants grew during the first few weeks of the experiment, but that the growth rates declined as the encroaching diatoms and epiphytes began to compete for light and nutrients.

Of the 120 *I. capensis* plants put on the two rafts in February, not a single one remained in March. Likewise, none of the *G. teedii* and *I. capensis* plants placed on the rafts in July and August respectively, managed to survive.

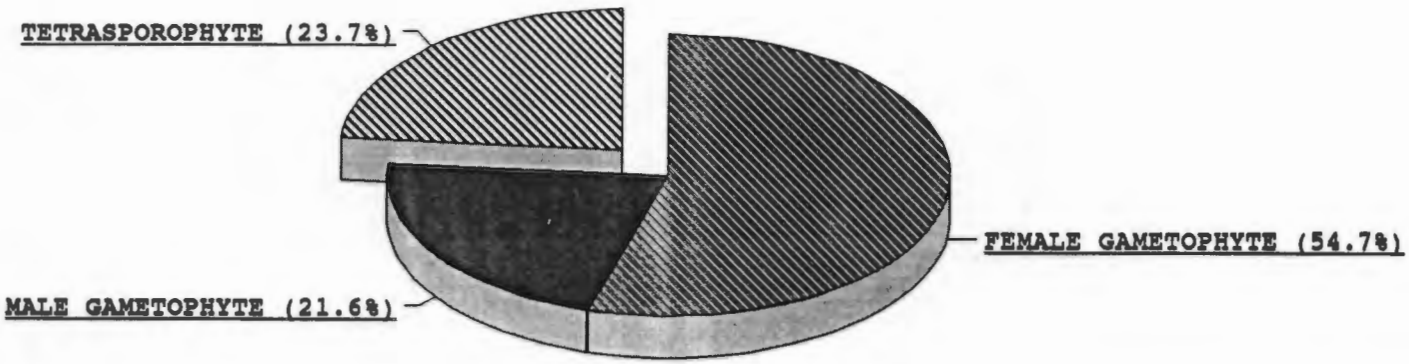
G. TEEDII EXPERIMENTS

Ratios of Life History Phases

Of the 2 391g of randomly collected *G. teedii* plants, 54.6% (or 1 305g) was easily identified as being female gametophyte by the presence of cystocarps. Of the remaining 45.4% of the plants, the resorcinol test indicated that 52.1% was tetrasporophyte, and 47.9% was male gametophyte. Therefore, 23.7% of the total sample was tetrasporophyte, 21.7% was male gametophyte, and 54.6% was female gametophyte (Figure 1).

On both the February and April visits to the Kraalbaai site, the *G. teedii* plants appeared healthy and abundant. However, on the third visit (July 1), the majority of the plants had died, but remained attached to the rocks as slimy white clumps that disintegrated when handled. On 19 July another trip was made to Kraalbaai, but despite searching more than 1km of coastline, not a single healthy-looking *G. teedii* plant could be located, although pieces of disintegrating thalli could still be found. A final trip was made to Kraalbaai on 27 August. It was then discovered that roughly 70% of all the rocky surface area at the

FIGURE 1. RATIO OF LIFE HISTORY PHASES OF G. TEEDII POPULATION IN FEBRUARY



low water mark was covered in a mat of *G. teedii* of no more than 5cm in length.

Carrageenan Content

The carrageenan contents of the gametophytic plants of *G. teedii* were slightly lower (mean of 35.4%) than those of the tetrasporophytes (mean of 39.7%). However, this difference was not significant at the 5% level.

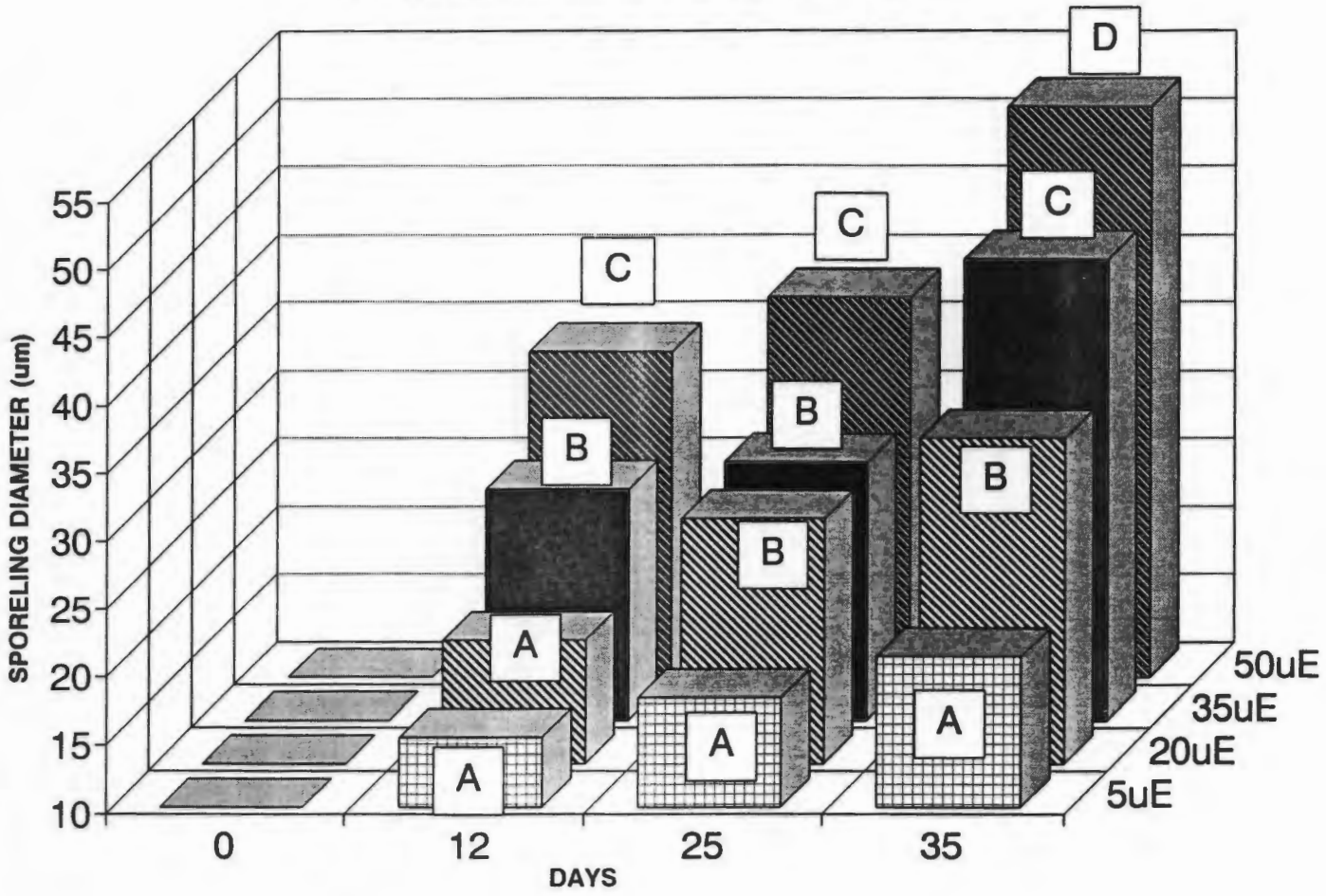
IRIDAEA EXPERIMENTS

Sporeling Growth Rates

Throughout the 5-week experiment, the *I. capensis* sporelings kept in 50 $\mu E.m^{-2}.sec^{-1}$ of light had significantly ($p<0.05$) larger diameters than the sporelings kept at the lower light intensities (Figure 2). The diameters of the 35 μE sporeling batch were significantly ($p<0.05$) larger than those of the 20 μE batch at the 12- and 35-day readings, while the sporelings kept in 5 & 20 $\mu E.m^{-1}.sec^{-1}$ did not have significantly different diameters ($p>0.05$).

The sporelings grown in 35 & 50 $\mu E.m^{-2}.sec^{-1}$ showed a significant ($p<0.05$) increase in diameter during the first 12 days, but did not grow significantly ($p>0.05$) over the following 13 days. However, towards the end of the experiment the growth rates of the 35 & 50 μE batches picked up once again, resulting in a significant difference ($p<0.05$) between between the 25- & 35-day readings. In contrast, the 5 & 25 μE sporeling batches showed a steadier, if more gradual, increase in diameter over the 5 weeks.

FIGURE 2. GROWTH RATES OF I. CAPENSIS SPORELINGS OVER 5 WEEKS



Note: The different letters indicate significant differences ($p < 0.05$) between values.

PHOTOSYNTHETIC RATES

The photosynthetic rate of *G. teedii* (at 190 $\mu E.m^{-2}.sec^{-1}$) increases significantly ($p<0.05$) from 10 to 15°C, peaks at 20°C, and then decreases rapidly (Figures 3.1 & 3.2). Thus, at 30°C photosynthesis barely exceeds respiration. The optimal temperature for photosynthesis in *I. capensis* lies between 10 & 15°C, although no significant differences ($p>0.05$) were found between the 10, 15, and 20°C readings. However, there was a significant drop ($p<0.05$) in the amount of oxygen evolved at temperatures higher than 20°C. In fact, at 30°C there was a negative net photosynthetic rate of -0.278 mg O_2.g dry wt^{-1}. hr^{-1}, suggesting that respiration was still occurring after the photosynthetic machinery had become impaired.

At 10°C, *I. capensis* photosynthesized at a significantly higher rate ($p<0.05$) than *G. teedii*, whereas at 20 and 25°C this situation was reversed. At 15°C there was no significant difference ($p>0.05$) between the amount of oxygen evolved from the two species.

The light response curves, performed at 15°C, were similar for *G. teedii* and *I. capensis*, such that no light intensity produced a significant difference ($p>0.05$) between the amount of oxygen evolved by the two species (Figure 4.1 & 4.2). The photosynthetic rates of both species increased significantly ($p<0.05$) from 0$\mu E.m^{-2}.sec^{-1}$ (where they respired but did not photosynthesize) to 150 μE. There were no significant differences ($p>0.05$) either within or between species for the light intensities from 150 to 300 $\mu E.m^{-2}.sec^{-1}$. *G. teedii* had an *Ik* value of 207 $\mu E.m^{-2}.sec^{-1}$, compared to 231 μE for *I.capensis*.

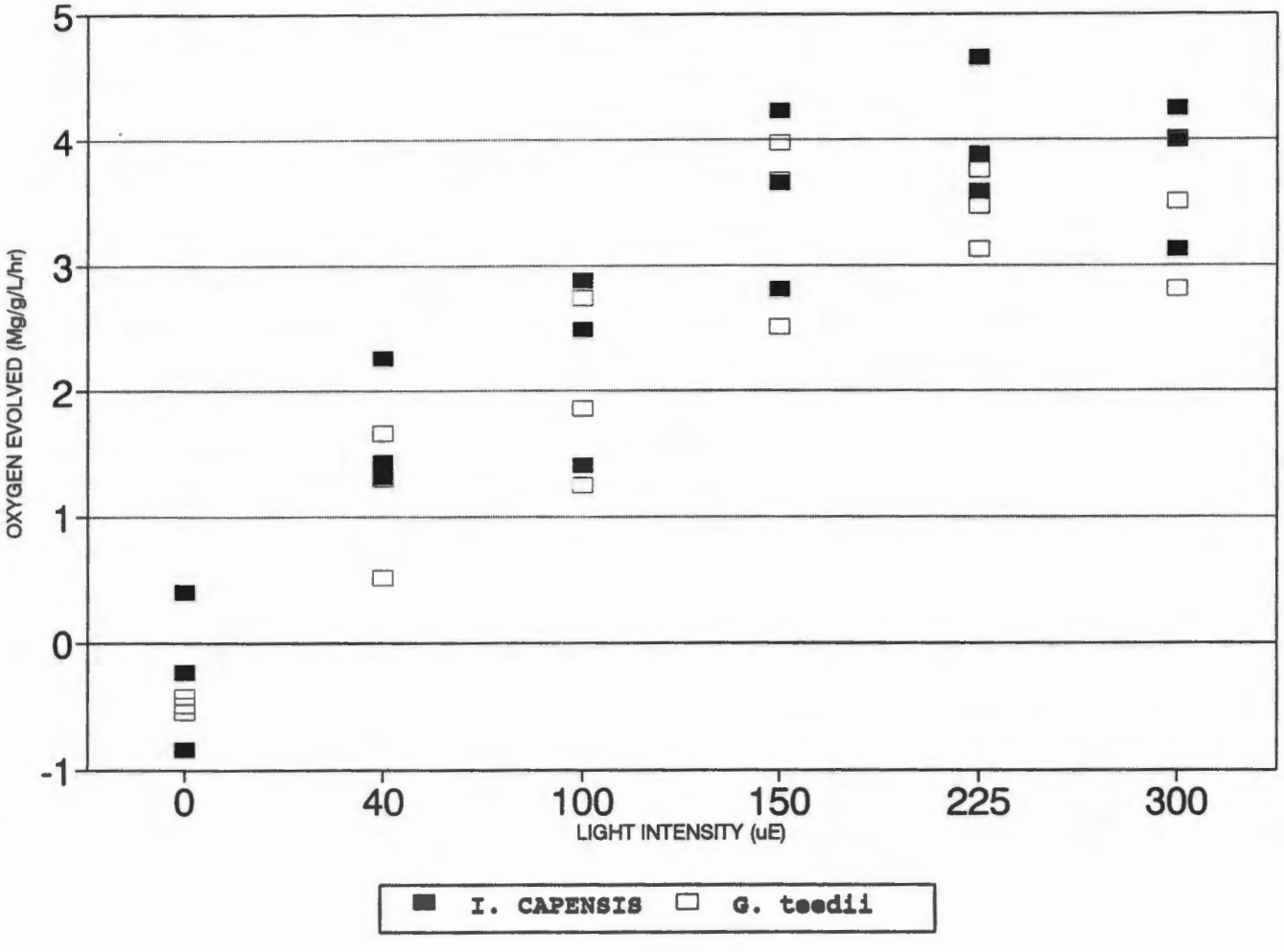


Figure 3.1 Plot of the three oxygen evolution values obtained over a range of light intensities for *Iridaea capensis* and *Gigartina teedii.*

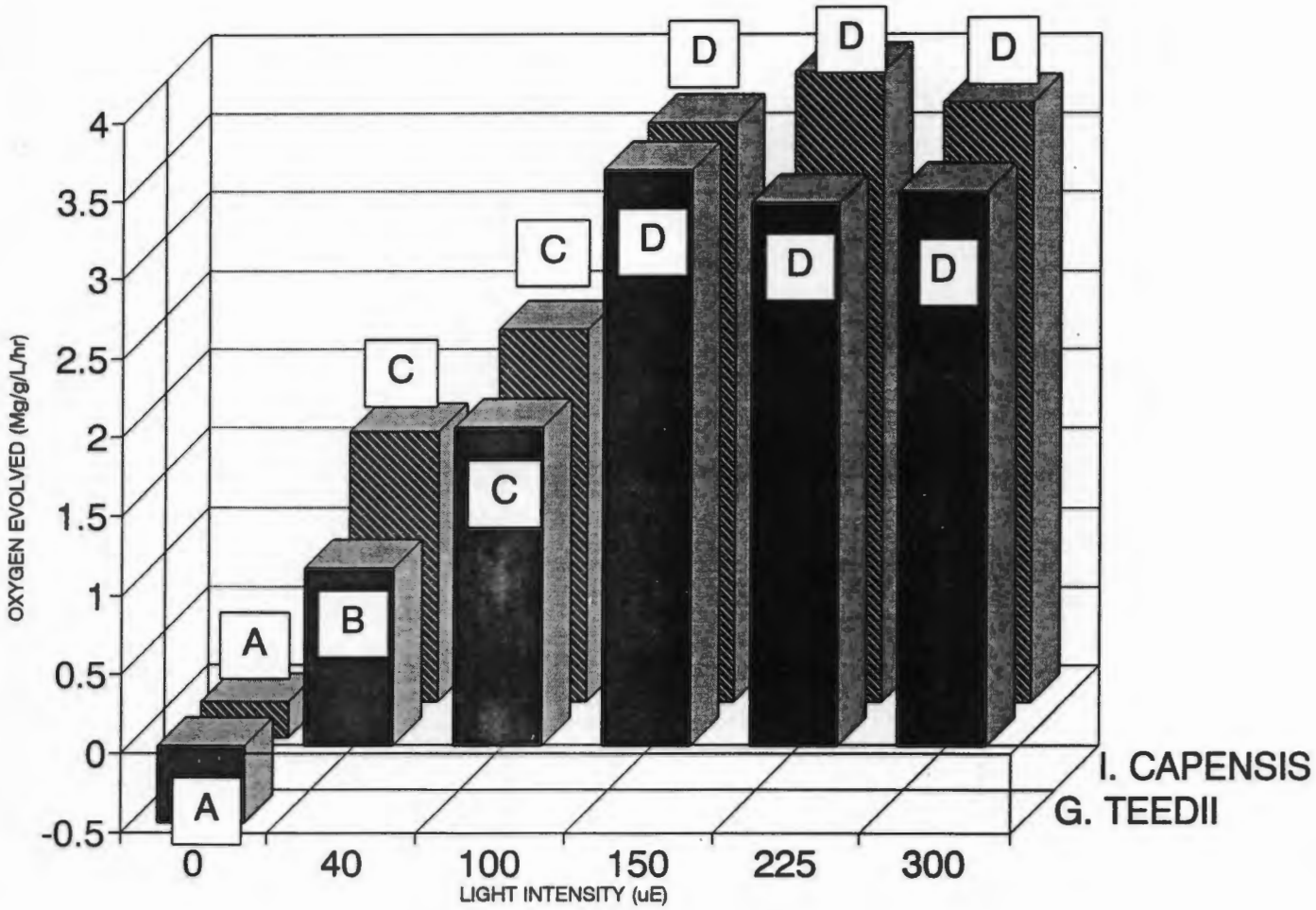


Figure 3.2 Bar graphs showing the averages of the *I. capensis* and *G. teedii* oxygen evolution values obtained at the various light intensities. The different letters indicate significant differences ($p < 0.05$) between values.

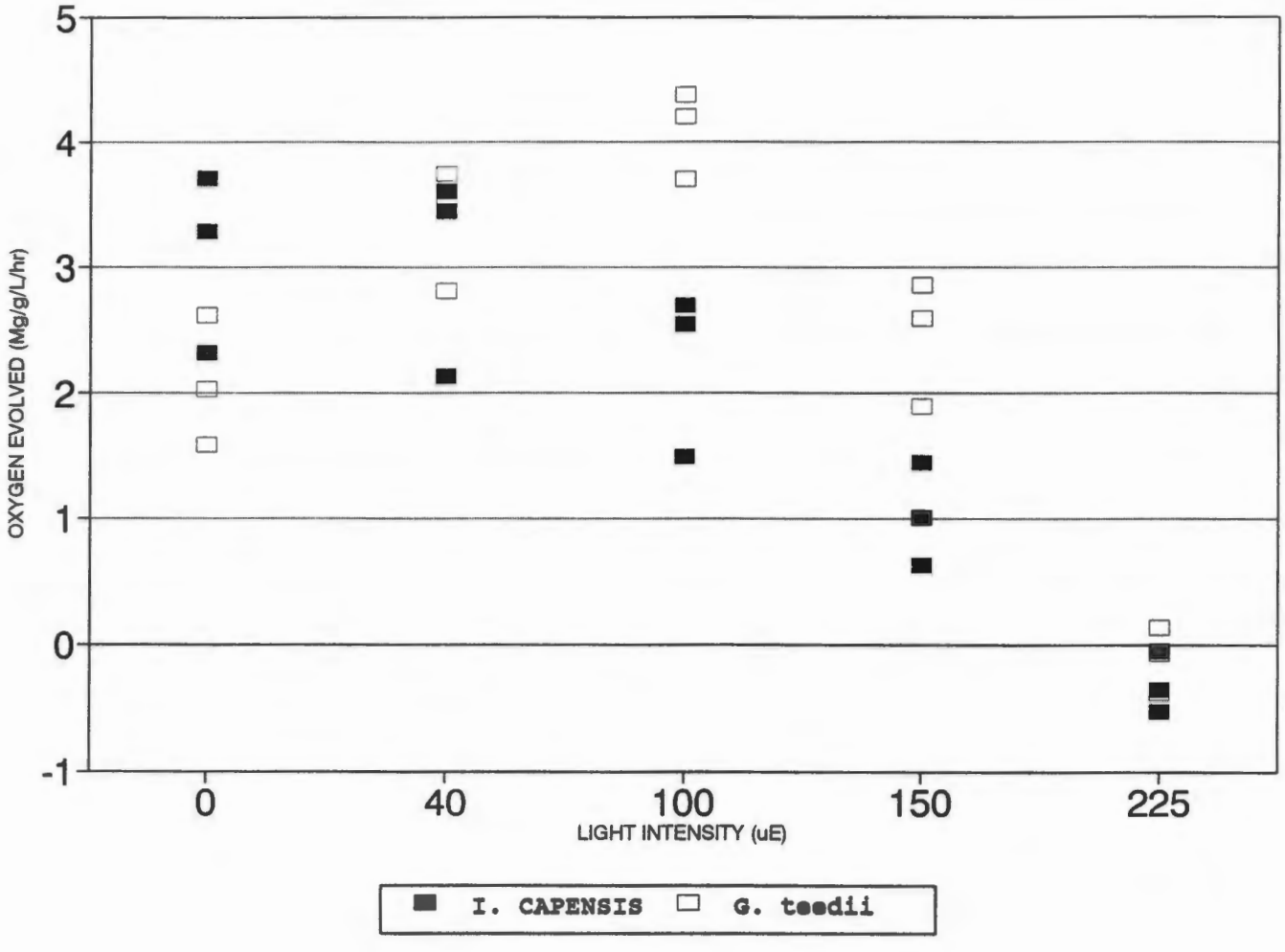


Figure 4.1 Plot of the three oxygen evolution values obtained over a range of temperatures for *Iridaea capensis* and *Gigartina teedii.*

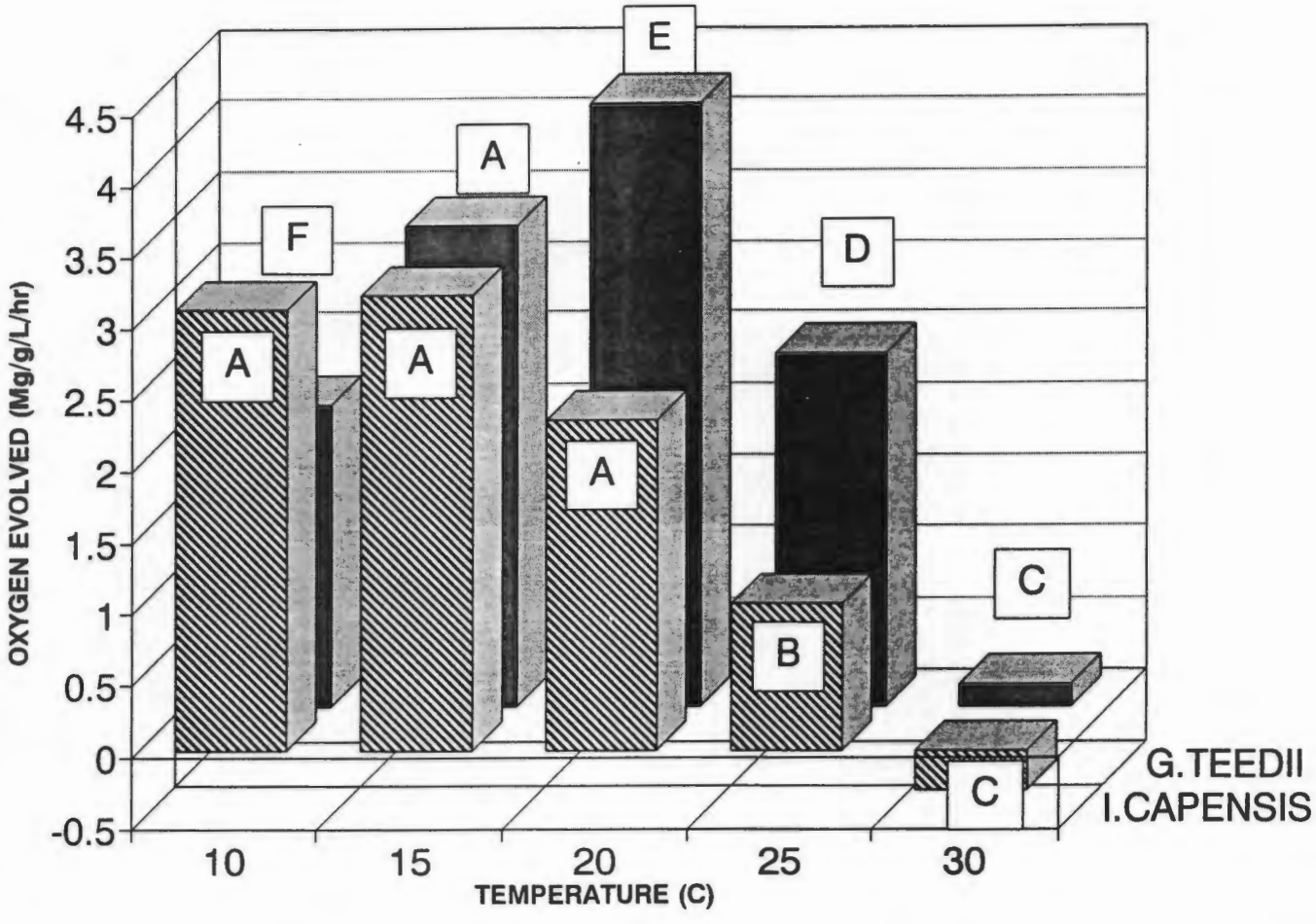


Figure 4.2 Bar graphs showing the averages of the *I. capensis* and *G. teedii* oxygen evolution values obtained at the various temperatures. The different letters indicate significant differences ($p < 0.05$) between values.

Discussion

In the Saldanha Bay mariculture experiments, *I. capensis* was not able to survive on the ropes, either in the summer or the winter. One possible reason for this is that the conditions on the rafts are unfavourable for this species. But since *I. capensis* has been observed growing naturally on the rafts in the past (G.J. Levitt, pers. com.) it must be assumed that the conditions are favourable for at least some of the year.

An alternative cause of death may be the method of attachment used - the braided ropes were opened and the stipes inserted into the line. With this method, the rope twists back into shape when it is suspended under tension to the raft, exerting pressure on the stipe. This may have irreparably damaged the stipes where they were in contact with the rope, ultimately causing the plants to break free of the raft. For this reason, when Waaland (1973) performed a similar mariculture experiment in the Pacific Northwest, with *Iridaea cordata*, he developed holders made from Tygon tubing. The plastic tubing permitted firm but gentle pressure on the holdfast and lower stipe portion of the plants, and the plants remained attached and healthy over the course of the experiment (March to September 1972).

The fact that *G. teedii* grew on the rafts in summer, but did not even survive in the winter, may be related to temperature requirements of this species. *G. teedii* photosynthesizes best at about 20°C (Figure 3), while the average daily temperature on the rafts wheh the summer experiment was being performed was 19.19°C (Sea Fisheries data, unpublished). However, the

photosynthetic rate of *G. teedii* at 10°C is half of the 20°C rate. Since the average daily temperature recorded on the rafts during the winter mariculture experiment was 13.22°C, the plants would have been photosynthesizing at well below their optimal rate. But it seems unlikely that low sea temperatures alone would kill *G. teedii*. What is more plausible is that its competitive ability is undermined at low temperatures, giving diatoms and other epiphytes a chance to overgrow it.

Another possible cause of death of the maricultured *G.teedii* plants in the winter may have been the unhealthy state of the parent population at that time of year. The plants were all collected at Kraalbaai on July 1, by which time the majority of the *G. teedii* population had already died. Naturally, only healthy-looking plants were selected for the mariculture experiment. But by the next visit to Kraalbaai (on July 19), even those *G. teedii* plants that had been healthy 18 days previously were now found to be dying. In other words, unfavourable environmental conditions (such as low temperatures) may have set in motion a gradual deterioration of the plants at Kraalbaai, so that those selected for mariculture in Saldanha Bay were already in a poor physiological condition.

Are there any other environmental factors besides low temperatures that might be causing the decline of *G. teedii* in the winter? It is clear that grazers are not the cause, since dead plants can be found clinging to the rocks and other seaweeds. Most of the area that the Kraalbaai population occupied in the summer became overgrown with *Sargassum* sp. in the winter, raising the question of whether *G. teedii* was being

deprived of light or space. However, there is a narrow band, about 1m on either side of the MLLW, where *Sargassum* sp. do not grow, but where *G. teedii* flourished in the summer and still perished in the winter. Since no large, shading seaweed species came to dominate this region of the shore during the decline of *G. teedii*, it can be concluded that a lack of light was probably not the cause of death.

Thus, there is no obvious evidence to suspect any environmental factor other than low sea temperature as the cause of the winter disappearance of *G. teedii*. Perhaps months of sub-optimal photosynthetic rates due to low temperatures undermines *G.teedii's* ability to compete with cold-adapted seaweeds, or reduces its ability to fight infection. It certainly seems unlikely that the Langebaan *G. teedii* population has adopted an annual life history, since *G. teedii* has been described by Guiry *et al.* (1987) as a perennial species that has been grown in culture for up to 10 years.

These authors also report successful growth experiments with excised tips of *G. teedii*. However, when a similar experiment was attempted for this project with plants collected in July, all the tips gradually lost colour and deteriorated, despite using filtered, autoclaved seawater. Therefore, it seems likely that these plants were already in a poor physiological state when they were collected at Kraalbaai. If *G. teedii* is ever to be seriously considered for its mariculture potential, it will be crucial to discover the exact cause of winter death. If, for example, their death is indeed temperature-related, then either a warmer mariculture site must be chosen, or cold-adapted strains

will have to be selected for.

The temperature response curves (Figure 3.2) reveal that the optimal temperature for photosynthesis in *G. teedii* is some 5°C higher than that of *I. capensis*. This is probably related to the fact that *G. teedii* has a mainly tropical and subtropical distribution (Guiry *et al.* 1987), while *I. capensis* is found in temperate waters from Cape Agulhas to Namibia (Bolton & Joska 1993). Furthermore, photosynthesis in *G. teedii* at 30°C still outweighed respiration, whereas the net photosynthetic rate of *I. capensis* was negative. Therefore, *I. capensis* would soon die at 30°C, while *G. teedii* will probably survive at this temperature. In fact, *G. teedii* from the Mediterranean survived a two-week culture experiment at 31°C, but not at 32°C (Guiry *et al.* 1987).

Since the temperature response curves were performed at 190 $uE.m^{-1}.sec^{-1}$ (the average of the two species' optimal light intensities) Figure 3.2 gives a good indication of the comparative productivities of the two species at different temperatures. At all temperatures from 15°C to 30°C *G. teedii* has the higher photosynthetic rate. Furthermore, the highest photosynthetic output that *I. capensis* can manage at this light intensity is 3.176 mg O_2.g dry $wt^{-1}.hr^{-1}$ (at 15°C), compared to 4.220 mgO_2.g dry $wt^{-1}.hr^{-1}$ for *G. teedii* at 20°C.

Why does *G. teedii* have the higher potential productivity? Perhaps the two most important parameters involved in determining a plant's photosynthetic rate are morphology and position on the shore. Levitt (1993) has reported that the average gross photosynthetic rate of the sublittoral seaweed species off the

Cape Peninsula is considerably less than that of the littoral species. For example, *G. polycarpa* in the sublittoral photosynthesizes at under half the rate of littoral *G. polycarpa* (3.6 *cf.* 7.9 g C.g $dw^{-1}.yr^{-1}$). However, since both the *G. teedii* and *I. capensis* plants were collected just above MLLW, this cannot account for the differences in photosynthetic rates observed in these two species.

The morphology of *G. teedii* and *I. capensis*, on the other hand, is extremely different. *G. teedii* has fine branches projecting from flattened main axes, giving it a greater surface area than the flattened, strap-like *I. capensis*. Therefore, *G. teedii* is probably more efficient at utilizing available light energy, nutrients, and carbon dioxide, thus accounting for its higher productivity than *I. capensis*. For example, Arnold & Murray (1980) also found that finely branched Green Algal species had higher light-saturated production rates than thicker, more structurally complex species.

Talling (1957, cited in Arnold & Murray 1980) introduced the quantity *Ik* to describe the transition between the phytochemical and the enzymatic portions of the photosynthesis-irradiance curve. In a study of five Cape littoral species, Levitt (1993) concluded that those with *Ik* values above 200μE.m^{-2}.sec^{-1} were sun-adapted. This seems to be a fitting description for *I.capensis* (*Ik*=230.9), especially since the plants collected for the photosynthetic experiment were from exposed rocks. The *G. teedii* plants, with their *Ik* value of 206.0μE.m^{-2}.sec^{-1}, also appear to have been sun-adapted, in agreement with the fact that they were collected from a bare strip of rock at MLLW.

The resorcinol test showed that the Kraalbaai *G. teedii* population was 23.7% tetrasporophyte in February. This figure is similar to the year-round 20% and 33% tetrasporophytes reported for *Gigartina stiriata* and *Gigartina polycarpa* respectively (Bolton & Levitt 1992). Since the gametophyte and tetrasporophyte carrageenan contents of *G. teedii* are similar (35.4% & 39.7% respectively), either life history phase could be used for a mariculture industry that was interested in carrageenan contents alone.

G. teedii is a member of the Gigartinaceae, and thus has *lambda*-family carrageenan in the tetrasporophyte generation, and *kappa*-family carrageenan in the gametophytic phase. Therefore, if the carrageenan industry were to be interested in *G. teedii* for a specific type of carrageenan (eg. *lambda*) then only tetrasporophytic strains need be maricultured. Such a situation occurs on Zanzibar, where the Danish carrageenan manufacturers Copenhagen Pectin are interested only in iota carrageenan, and therefore only *Eucheuma denticulatum* (an *iota*-containing species) is maricultured (personal observation). But just identifying tetrasporophytic plants of *G. teedii* will be an arduous task, since the resorcinol test will have to be used, and only about a quarter of all plants tested will be tetrasporophytes. Since a large number of individuals is necessary if strain selection is to be performed, thousands of plants will have to be screened.

Figure 2 shows that *I. capensis* sporelings kept in 50μE. $m^{-2}.sec^{-1}$ were always significantly larger ($p<0.05$) than those kept at lower light intensities, suggesting that the saturating light intensity for sporeling growth may be much higher than 50 μE.m-

^{2}.sec^{-1}. This result is quite unexpected, since most sporelings require less than 50μE.m^{-2}.sec^{-1} of light for optimal growth. For example, Levitt (unpublished) found 27 μE.m^{-2}.sec^{-1} to be the saturating light intensity for *Gigartina polycarpa* sporeling growth.

However, Waaland (1973) found that *Iridaea cordata* plants of 2.5mm length grew best (at 14°C) in 243 μE.m^{-2}.sec^{-1} of light, or, according to that author "a light intensity substantially higher than those used for routine laboratory cultivation of most marine red algae, many of which are killed by such high light intensities". Thus, if *I. capensis* sporelings and juvenile *I.cordata* plants both have unusually high light preferences for optimal growth, one can only wonder whether this is not a trait shared by other species of this genus.

Once the *I. capensis* sporelings that had been established on the ropes had produced uprights between 1.0 and 1.6mm, they were placed on the rafts in Saldanha. However, these ropes soon became overgrown with diatoms, *Enteromorpha* sp. and *Ceramium* sp., which may have been the cause of death of the sporelings. What factors may be in operation to enable *I. capensis* juveniles to establish successfully in a seemingly hazardous environment such as a rocky shore, but not on a rope in Saldanha Bay? The sea temperature in winter (the time of recruitment for *I. capensis*) is most likely the same on the shore as it is in Saldanha Bay, while light should not be a limiting factor on the rafts. Perhaps it is wave action that prevents a mat of diatoms forming on the rocks as it does on the rafts. Or maybe low tides kill diatoms and other opportunistic epiphytes through desiccation.

Alternatively, the *I. capensis* juveniles may have simply been too small to survive the translocation to the rafts. In Japan, *Laminaria japonicum* ("kombu") seedlings are only outplanted when they have reached 1.0-1.5cm in length (Kawashima 1993). Whatever the reason for the failure of *I. capensis* juveniles to grow on the rafts, it is likely that success could be achieved with further experimentation. For instance, Waaland (1975) has already shown that it is possible to mariculture *I.cordata* in the Pacific Northwest by seeding ropes with spores. After culturing the sporelings for 3 months at 100 $uE.m^{-2}.sec^{-1}$ of light, the blades had reached a length of 1-2cm. They were then outplanted to a raft at a depth of 1m, where they reached an average size of 100 g after 3 months.

Therefore, to obtain 100 grams of maricultured *I. cordata*, one would not only have to perform the intricate task of seeding ropes, but also wait up to six months before the plants had reached an adequate size. By comparison, vegetative fragments of *Eucheuma denticulatum* maricultured in Zanzibar can triple in size in a mere 3 weeks. So it is little wonder that Waaland's discoveries were never put into practice. One should remember, though, that Waaland was devising his technique for the mariculture of *I. cordata* in the early 1970's, about the same time that Maxwell Doty was inventing his extremely successful mariculture technique for *Eucheuma* in the Philippines. In other words, Waaland was not to know that the carrageenan market was about to be flooded with cheap, vegetatively maricultured *Eucheuma*.

It seems likely that mariculture techniques involving spore

establishment on ropes will remain confined to the seaweed food mariculture industry. The reason for this is that seaweeds sold as food always fetch much higher prices than those used for the extraction of phycocolloids. For example, brown algae used as food in the Far East sell for US$ 7,000-10,0000 per dry tonne, compared to US$ 150-500 for brown algae sold for the extraction of algin (McHugh 1984). In other words, only when the end-product can fetch a considerable price is the costly and lengthy sexual method for seaweed mariculture worthwhile.

However, there may be one possible role for spore-based mariculture of cold water carrageenophytes. Certain species of *Gigartina*, for example, possess perennial holdfasts which can grow horizontally over the substrate, thereby increasing the surface area from which new blades can arise. Thus, by seeding ropes with these species of *Gigartina*, it may be possible to produce ropes that are entirely covered by perennial holdfasts. Once these ropes are on the rafts, mature blades can be harvested without having to re-seed the ropes, since the perennial holdfast will continue to produce new recruits.

In fact, it may not be necessary to seed the ropes at all. Waaland (1975) found that by inserting juvenile (2-5cm long) *Gigartina exasperata* plants into braided ropes on rafts, the blades formed holdfasts which enveloped the ropes and began producing upright blades during the next growing season. *Iridaea cordata* plants, however, did not form holdfasts when inserted into the ropes (Waaland 1975). This may be because *Iridaea* has an epilithic crustose phase, but not a spreading holdfast (Bolton & Joska 1993).

Therefore, there are two important directions in which this project should be extended in the future. The first is to test whether South African species of *Gigartina* can be encouraged to form their perennating holdfasts on ropes. This can be attempted either through seeding the ropes with spores, or by inserting the holdfasts of species such as *G. polycarpa* and *G. stiriata* between the braids of the ropes (either in the laboratory or in the field). If these techniques prove successful, then the economic viability should be assessed in terms of grams of biomass produced per m^2 per day. Unless this figure proves to be comparable with thse of *Eucheuma* farms in the tropics, then this mariculture technique will not be viable. A potential problem associated with growing *Gigartina* from perennating holdfasts is that there is likely to be a seasonal pattern of recruitment, thereby limitting the number of harvests each year. For example, both *G. polycarpa* and *G. stiriata* have their major recruitment period in the winter (Bolton & Levitt 1992).

The second, and most important, future direction for this project should be to screen South African carrageenophyte species for their asexual reproductive potential. But what traits should be used as a broad indicator of mariculture potential? Perhaps it is best to examine the similarities that exist between the successfully maricultured genera *Eucheuma* and *Gracilaria*. Species of both genera tend to be multiaxial and highly branched. Many *Gracilaria* populations are known to reproduce asexually, with fragments being dispersed during storms, and later becoming embedded in the substratum where they regenerate (Trono 1993). Collen & Pedersen (1992) have suggested that the disease in

Eucheuma known as "ice-ice" is merely the plant's response to unfavourable environmental conditions, causing fragmentation of the thallus so that branches might be dispersed to more favourable habitats.

However, if a species is to employ fragmentation as a dispersal mechanism, it must not only be able to rapidly repair snapped branches, but also to readily produce new branches that grow rapidly from the tips. Since *Gracilaria* spp. possess these abilities (A.J. Smit, pers. com.), as do *Eucheuma* spp. (Azanza-Corrales & Dawes 1989), it is not surprising that species of these two genera are easily cultured in the laboratory.

So in the context of South African carrageenophytes, what criteria should be used for screening potential species? It seems logical to begin with branched species, since they should be capable of growing in all directions, while blade-like species are restricted to two-dimensional growth. Also, branched species will grow mainly in the tips, so there will be a large number of growth regions. Once such carrageenophytes have been identified, screening can take place either in the laboratory or in the field. By culturing small fragments, it should be possible to determine a species' ability to repair broken off branches and to produce new branches. In addition, temperature preferences and maximum growth rates can be determined.

The alternative is to test species directly for their mariculture potential, i.e. by attaching the plants to ropes on rafts. This method may be more practical, especially since there are a limited number of sheltered sites along the South African coastline. In other words, a bay or estuary that seems to have

mariculture potential should be picked, and species then tested for their ability to grow at that location. Local feasibilty studies of this sort will not only serve to identify suitable areas for mariculture, but also to find fast-growing strains suited to a particular species.

In spite of the rather poor performance of *G. teedii* on the Saldanha Bay rafts, experiments with this species must certainly continue. The morphology of *G. teedii* fits the criteria for mariculture proposed above, while Guiry *et al.* (1987) have shown that excised tips are readily cultured. Furthermore, the Saldanha Bay experiments have shown that this species can tolerate being inserted into rope. Perhaps Saldanha Bay is an unsuitable site for mariculturing *G. teedii*, being too cold in the winter, and suffering from a thermocline in the summer (R.J. Anderson, pers. com.). Since *G. teedii* has also been found in Algoa Bay, West *s.n.* 1958 (Bol), an attempt should certainly be made to mariculture it there. Not only is the sea temperature in Algoa Bay much warmer, having a summer and winter average of 16.32 and 20.67°C respectively (Port Elizabeth municipality), but there is a relatively protected area northeast of the harbour wall which is suitable for mariculture rafts (Aken *et al.* 1993).

Another carrageenophyte worth investigating is *Hypnea spicifera*, a highly branched species which contains pure *K*-carrageenan (Furneaux & Miller 1986) and dominates areas of the lower shore and subtidal fringe in the eastern Cape Province (Isaac & Hewitt 1953). In fact, harvesting of natural stocks of this species began on a small scale in 1988 (Anderson *et al.* 1989). Due to its distribution, mariculture experiments with

H.spicifera ought also be initiated in Algoa Bay.

For Saldanha Bay, the branched carrageenophyte *Gymnogongrus polycladus* seems a good candidate for mariculture experiments. *Gymnogongrus* spp. contain *K*-family carrageenans, and are commercially harvested in Chile (Bolton & Levitt 1992). *G.polycladus* grows abundantly in the upper subtidal near Skaapeneiland, towards the mouth of Langebaan Lagoon (personal observation). This area is much more exposed than Kraalbaai, making the conditions there similar to those experienced in Saldanha Bay. Perhaps as a result, the branches of G. polycladus are very hardy, which can be an important trait for mariculture. Even the thickly branched *Eucheuma* spp. maricultured in Zanzibar lose considerable biomass during on windy days (personal observation).

In conclusion, this study has highlighted some of the most important challenges that exist when trying to mariculture seaweeds for phycocolloids. Prices dictate that vegetative mariculture methods are the most feasible. Therefore, excised tips of all branched carrageenophytes should be placed in laboratory culture to find those that grow and branch rapidly, and show effective repair of damaged tissue. Of those species that fare best in culture, large numbers of plants from diverse populations must be placed on rafts in the nearest sheltered waters. In this way, it should be possible to isolate strains best suited to rope mariculture, which can then be propagated vegetatively to produce the stock from which a future carrageenan mariculture industry might be established.

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